TAB 1

This appendix contains a glossary of symbols, acronyms, abbreviations and terms used in the text of this methods development guide

4.1 Glossary of Symbols, Acronyms and Abbreviations

CE	Capillary Electrophoresis
CZE	Capillary Zone Electrophoresis
cm	Centimeter, one hundredth of one meter, 10^{-2} meters
DNA	Deoxyribonucleic Acid
DTAB	Dodecyltrimethylammonium bromide
EOF	Electroosmotic Flow
FSCE	Free Solution Capillary Electrophoresis
HCl	Hydrochloric Acid
HV	High Voltage
i.d.	Internal Diameter
IEF	Isoelectric Focusing
Inj	Injection
Inc	Increment
kV	Kilo Volt, one thousand volts, 10^{3} volts
μ	Mobility
μA	Microampere, Microamp, one millionth of one ampere, 10^{-6} amperes
μ_{app}	Apparent mobility
μ_e	Electrophoretic mobility
μ_{eo}	Electroosmotic mobility
μm	Micrometer, Micron, one millionth of one meter, 10^{-6} meters
M	Mole, molar solution, a solution containing one molecular weight equivalent in grams/liter (see Normal)
min or m	Minute
MECC	Micellar Electrokinetic Capillary Chromatography
mg	Milligram, one thousandth of one gram, 10^{-3} grams
mL	Milliliter, one thousandth of one liter, 10^{-3} liters
mM	Millimole, one thousandth of one Mole, 10^{-3} moles
N	Normal solution, a solution containing one molecular weight equivalent in grams/liter, (see M, molar)
NaOH	Sodium Hydroxide
nm	Nanometer, one billionth of one meter, 10^{-9} meter

4.2 Glossary of Terms

Apparent Mobility

The apparent mobility of a molecule is the vector sum of the electrophoretic mobility and the electroosmotic flow, assuming that there are no wall interactions

Buffer

The electrolyte used as the conductive medium in electrophoresis. A solution of a weak acid and its conjugate weak base in which the pH remains relatively stable with the addition of other acids or bases.

Charge Reversal Reagent

A reagent used to reverse the charge, from negative to positive, on the surface of a fused silica capillary The charge reversal allows the analysis of positively charged molecules by reducing any wall interactions. (see MicroCoat™)

Deprotonated

When a positively charged molecule (having excess protons) becomes neutral or negatively charged

Electrokinetic Injection

Introduction of the sample into the capillary by electromigration. The end of the capillary is placed into the sample vial and an electric field is applied Charged molecules will migrate towards the electrode of opposite charge and, with the appropriate electrode configuration, into the capillary. Electrokinetic injection can, by manipulating the charge on various components within a sample and the configuration of the electrodes, be used either for selective injection or as a concentration step.

Electroosmotic Flow

The bulk flow of liquid under the influence of an electric field, caused by the interaction between ions in solution and the charged surface of the capillary The charged capillary surface causes a build-up of counterions in the liquid, close to the capillary surface. When an electric field is

applied, these counterions will be attracted towards the electrode of opposite charge causing the liquid to flow in that direction. In an untreated fused silica capillary, the surface will have a negative charge, the attracted counterions will be positive and the flow will be towards the negative electrode. The electroosmotic flow is proportional to the charge on the surface of the capillary and the applied electric field.

Electropherogram

The graphical representation of the migration pattern of solute molecules through a detector. Equivalent to a chromatogram in chromatography.

Electrophoresis

A separation technique based on the mobility of molecules under the influence of an electric field

Electrophoretic Mobility

The mobility of molecules, in solution, under the influence of an applied electric field The electrophoretic mobility in free solution electrophoresis, assuming that there are no wall interactions, is influenced by the size and shape of the molecule (frictional coefficients), the net charge on the molecule and the electric field strength

Free Solution Electrophoresis

Electrophoresis carried out in a liquid phase without the use of anticonvective or sieving matrices.

Hydrostatic Injection

Introduction of the sample into the capillary by pressure differential. Can be positive pressure (pumping) or negative pressure (vacuum)

Ion

An atom, or radical, which is charged as a result of either gaining (cation) or losing (anion) one or more electrons.

Ionic

The state of a molecule containing one or more ions (charged), can be cationic (-) or anionic (+) The net charge on a zwitterionic molecule is pH

TAB 2

McGraw-Hill DICTIONARY OF SCIENTIFIC AND TECHNICAL TERMS

Fourth Edition

Sybil P. Parker
EDITOR IN CHIEF

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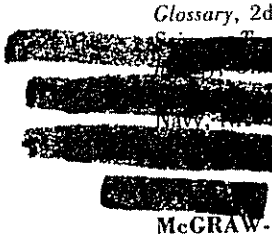
TB 4

On the cover: Pattern produced from white light by a computer-generated diffraction plate containing 529 square apertures arranged in a 23 × 23 array. (R. B. Hoover, Marshall Space Flight Center)

On the title pages: Aerial photograph of the Sinai Peninsula made by Gemini spacecraft. (NASA)



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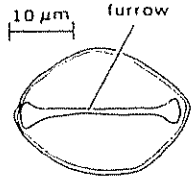
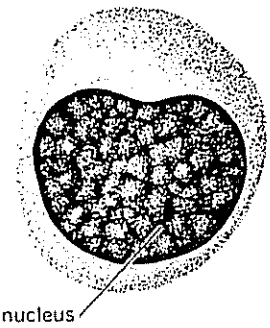
nonocolpate pollen [BOT] Pollen grains having a single furrow { ¦mänˌōˈkōlˌpāt ˌpälˌən }
nonocoque [AERO ENG] A type of construction as of a rocket body, in which all or most of the stresses are carried by the skin { ˈmänˌōˌkäk }
nonocord switchboard [ELEC] Local battery switchboard in which each telephone line terminates in a single jack and plug { ˈmänˌōˌkȯrd ˈswichˌbȯrd }
nonocotyledon [BOT] Any plant of the class Liliopsida; all have a single cotyledon { ¦mänˌōˌkädˌəlˈēdˌən }
nonocotyledoneae [BOT] The equivalent name for Liliopsida { ˌmänˌōˌkädˌəlˌəˈdōnēˌē }
nonocrepid [INV ZOO] A desma formed by secondary deposits of silica on a monaxon { ¦mänˌōˌkrepˌəd }
nonocular vision [MED] Sight with one eye { məˈnäkˌyələr ˈvizhˌən }
Nonocyathea [PALEON] A class of extinct parazoans in the phylum Archaeocyatha containing single-walled forms { ˌmänˌōˌsīˈāˌthēˌə }
nonocyte [HISTOL] A large (about 12 micrometers), agranular leukocyte with a relatively small, eccentric, oval or kidney-shaped nucleus { ˈmänˌōˌsīt }
nonocytic angina *See* infectious mononucleosis { ¦mänˌōˌsidˌikˈanˌjəˌnə }
nonocytic leukemia [MED] A form of leukemia in which monocytic cells are predominant in the blood. Also known as myelomonocytic leukemia { ¦mänˌōˌsidˌik lüˈkēˌmēˌə }
monocytoma [MED] A neoplasm composed principally of monocytes, usually anaplastic { ¦mänˌōˌsīˈtōˌmə }
monocytopenia [MED] Reduction in the number of circulating monocytes per unit volume of blood to below the minimum normal levels { ˌmänˌōˌsīdˌəˈpēˌnēˌə }
monocytosis [MED] Increase in the number of circulating monocytes per unit volume of blood to above the maximum normal levels { ˌmänˌōˌsīˈtōˌsəs }
monodactylous [ZOO] Having a single digit or claw { ¦mänˌəˈdakˌtəˌləs }
Monodellidae [INV ZOO] A monogeneric family of crustaceans in the order Thermosbaenacea distinguished by seven pairs of biramous pereiopods on thoracomeres 2–8, and by not having the telson united to the last pleonite { ¦mänˌəˈdelˌəˌdē }
monodelphic [VERT ZOO] 1. Having a single genital tract, in the female. 2. Having a single uterus { ¦mänˌōˈdelˌfik }
monodisperse colloidal system [CHEM] A colloidal system in which the suspended particles have identical size, shape, and interaction { ¦mänˌōˌdiˈspərs kəˈlȯidˌəl ˈsisˌtəm }
monodispersity [ORG CHEM] Polymer system that is homogeneous in molecular weight, that is, it does not have a distribution of different molecular-weight chains within the total mass { ¦mänˌōˌdiˈspərˌsəd ē }
monodromy theorem [MATH] If a complex function is analytic at a point of a bounded simply connected domain and can be continued analytically along every curve from the point, then it represents a single-valued analytic function in the domain { ˈmänˌəˌdrōˌmē ˌthirˌəm }
monoecious [BOT] 1. Having both staminate and pistillate flowers on the same plant. 2. Having archegonia and antheridia on different branches. [ZOO] Having male and female reproductive organs in the same individual. Also known as hermaphroditic { məˈnē shəs }
Monoedidae [INV ZOO] An equivalent name for Colydiidae { məˈnēˌdəˌdē }
monoelectron oscillator *See* geonium { ¦mänˌōˌiˈlekˌträn ˈäsˌəˌlādˌər }
monoenergetic gamma rays [PHYS] A beam of gamma rays whose energies are confined to an extremely narrow range { ¦mänˌōˌenˌərˈjedˌik ˈgamˌə ˌrāz }
monoester [ORG CHEM] An ester that has only one ester group { ¦mänˌōˈesˌtər }
monofier [ELECTR] Complete master oscillator and power amplifier system in a single evacuated tube envelope; electrically, it is equivalent to a stable low-noise oscillator, an isolator, and a two- or three-cavity klystron amplifier { ˈmänˌəˌfīˌər }
monofilament [TEXT] A single, large, continuous filament (single-strand thread) of a natural or synthetic fiber { ¦mänˌəˈfilˌəˌmənt }
monofuel propulsion [AERO ENG] Propulsion system which obtains its power from a single fuel; in rocket units, the fuel furnishes both oxygen supply and the hydrocarbon for combustion { ˈmänˌōˌfyül prəˈpəlshən }
monogamy [ANTHRO] Marriage to only one person at a time { məˈnägˌəˌmē }
monogastric [VERT ZOO] Having only one digestive cavity { ¦mänˌōˈgasˌtrik }
Monogenea [INV ZOO] A diverse subclass of the Trematoda which are principally ectoparasites of fishes; individuals have enlarged anterior and posterior holdfasts with paired suckers anteriorly and opisthaptors posteriorly { ˌmänˌəˈjēˌnēˌə }
Monogenoidea [INV ZOO] A class of the Trematoda in some systems of classification; equivalent to the Monogenea of other systems { ˌmänˌəˌjəˈnȯidˌēˌə }
monogeosyncline [GEOL] A primary geosyncline that is long, narrow, and deeply subsided; composed of the sediments of shallow water and situated along the inner margin of the borderlands { ¦mänˌōˌjēˌōˈsinˌklīn }
monoglyceride [ORG CHEM] Any of the fatty-acid glycerol esters where only one acid group is attached to the glycerol group, for example, $RCOOCH_2CHOHCH_2OH$; examples are glycerol monostearate and monolaurate; used as emulsifiers in cosmetics and lubricants { ¦mänˌōˈglisˌəˌrīd }
Monogonota [INV ZOO] An order of the class Rotifera, characterized by the presence of a single gonad in both males and females { ˌmänˌōˌgōˈnädˌə }
monogony [BIOL] Asexual reproduction { məˈnägˌəˌmē }
monogynous [BOT] Having only one pistil. [VERT ZOO] 1. Having only one female in a colony. 2. Consorting with only one female { məˈnäjˌəˌnəs }
monohybrid [GEN] A hybrid individual heterozygous for one gene or a single character { ¦mänˌōˈhīˌbrəd }
monoid [MATH] A semigroup which has an identity element { ˈmäˌnȯid }
monoideism [PSYCH] A mental condition marked by the domination of a single idea; persistent and thorough preoccupation with one idea, but seldom an idea that is complete { ¦mänˌōˈidˌēˌizˌəm }
monolayer *See* monomolecular film { ˈmänˌōˌlāˌər }
monolinuron *See* 3-(4-chlorophenyl)-1-methoxyl-1-methylurea { ˌmänˌəˈlinˌyəˌrän }
monolith [MATER] A large concrete block { ˈmänˌəˌlith }
monolithic [CIV ENG] Pertaining to concrete construction which is cast in one jointless piece. [SCI TECH] Constructed from a single crystal or other single piece of material { ˌmänˌəˈlithˌik }
monolithic ceramic capacitor [ELECTR] A capacitor that consists of thin dielectric layers interleaved with staggered metal-film electrodes; after leads are connected to alternate projecting ends of the electrodes, the assembly is compressed and sintered to form a solid monolithic block { ˌmänˌəˈlithˌik səˈramˌik kəˈpasˌəd ər }
monolithic filter [COMMUN] A device used to separate telephone communications sent simultaneously over the transmission line, consisting of a series of electrodes vacuum-deposited on a crystal plate so that the plated sections are resonant with ultrasonic sound waves, and the effect of the device is similar to that of an electric filter { ˌmänˌəˈlithˌik ˈfilˌtər }
monolithic integrated circuit [ELECTR] An integrated circuit having elements formed in place on or within a semiconductor substrate, with at least one element being formed within the substrate { ˌmänˌəˈlithˌik ˈintˌəˌgrād ˌəd ˈsərˌkət }
monomer [CHEM] A simple molecule which is capable of combining with a number of like or unlike molecules to form a polymer; it is a repeating structure unit within a polymer { ˈmänˌəˌmər }
monomial [MATH] A polynomial of degree one { məˈnōˌmēˌəl }
monomineralic [PETR] Of a rock, composed entirely or principally of a single mineral { ¦mänˌōˌminˌəˈralˌik }
Monommidae [INV ZOO] A family of coleopteran insects in the superfamily Tenebrionoidea { məˈnamˌəˌdē }
monomolecular film [PHYS CHEM] A film one molecule thick. Also known as monolayer { ¦mänˌōˌməˈlekˌyələr ˈfilm }
monomorphic [BIOL] Having or exhibiting only a single form { ¦mänˌəˈmȯrfik }

MONOCOLPATE POLLEN



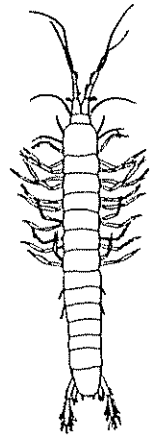
Monocolpate pollen of *Zamia floridana*

MONOCYTE



Diagrammatic representation of a monocyte

MONODELLIDAE



Drawing of a male *Monodella halophila* Karaman showing the seven pairs of appendages and the characteristic telson. *(After S. Karaman)*

TAB 3

CHAPTER 1

Background Theory and Principles of Capillary Electrophoresis

1 Introduction

This chapter describes the basic theoretical concepts and principles of capillary electrophoresis (CE). The depth of discussion should provide enough background to understand the basic operation of CE instruments and the principles by which CE separates analytes. This is complemented in the next chapter by a discussion of the most common modes of separation operated by CE. For a more comprehensive explanation of the theoretical aspects of CE, please refer to one or more of the reference books listed in the Bibliography. In addition, a list of definitions for terms and abbreviations is given in the Glossary.

2 What is Capillary Electrophoresis?

The process of electrophoresis is defined as 'the differential movement or migration of ions by attraction or repulsion in an electric field'. In practical terms, a positive (anode) and negative (cathode) electrode are placed in a solution containing ions. Then, when a voltage is applied across the electrodes, solute ions of different charge, *i.e.*, anions (negative) and cations (positive), will move through the solution towards the electrode of opposite charge. Capillary electrophoresis, then, is the technique of performing electrophoresis in buffer-filled, narrow-bore capillaries, normally from 25 to 100 μm in internal diameter (ID).

3 Instrumentation

The instrumentation required for CE is remarkably simple in design, as Figure 1.1 illustrates. The ends of a capillary are placed in separate buffer reservoirs, each containing an electrode connected to a high-voltage power supply capable of delivering up to 30 kV. The sample is injected onto the capillary by temporar-

differences in the speed of migration (migration velocity) of ions or solutes. Now, ion migration velocity can be expressed as:

$$v = \mu_e E \tag{1.1}$$

where v is ion migration velocity ($m\,s^{-1}$), μ_e is electrophoretic mobility ($m^2\,V^{-1}\,s^{-1}$) and E is electric field strength ($V\,m^{-1}$).

The electric field strength is a function of the applied voltage divided by the total capillary length. Electrophoretic mobility is a factor that indicates how fast a given ion or solute may move through a given medium (such as a buffer solution). It is an expression of the balance of forces acting on each individual ion; the electrical force acts in favour of motion and the frictional force acts against motion. Since these forces are in a steady state during electrophoresis, electrophoretic mobility is a constant (for a given ion under a given set of conditions). The equation describing electrophoretic mobility is:

$$\mu_e = \frac{q}{6\pi\eta r} \tag{1.2}$$

where q is the charge on the ion, η is the solution viscosity and r is the ion radius. The charge on the ion (q) is fixed for fully dissociated ions, such as strong acids or small ions, but can be affected by pH changes in the case of weak acids or bases. The ion radius (r) can be affected by the counter-ion present or by any complexing agents used. From equation (1.2) we can see that differences in electrophoretic mobility will be caused by differences in the charge-to-size ratio of analyte ions. Higher charge and smaller size confer greater mobility, whereas lower charge and larger size confer lower mobility.

Electrophoretic mobility is probably the most important concept to understand in electrophoresis. This is because electrophoretic mobility is a characteristic property for any given ion or solute and will always be a constant. What is more, it is the defining factor that decides migration velocities. This is important, because different ions and solutes have different electrophoretic mobilities, so they also have different migration velocities at the same electric field strength. It follows that, because of differences in electrophoretic mobility, it is possible to separate mixtures of different ions and solutes by using electrophoresis.

5 Electroosmotic Flow (EOF)

A vitally important feature of CE is the bulk flow of liquid through the capillary. This is called the electroosmotic flow and is caused as follows.

An uncoated fused-silica capillary tube is typically used for CE. The surface of the inside of the tube has ionisable silanol groups, which are in contact with the buffer during CE. These silanol groups readily dissociate, giving the capillary wall a negative charge. Therefore, when the capillary is filled with buffer, the negatively charged capillary wall attracts positively charged ions from the buffer

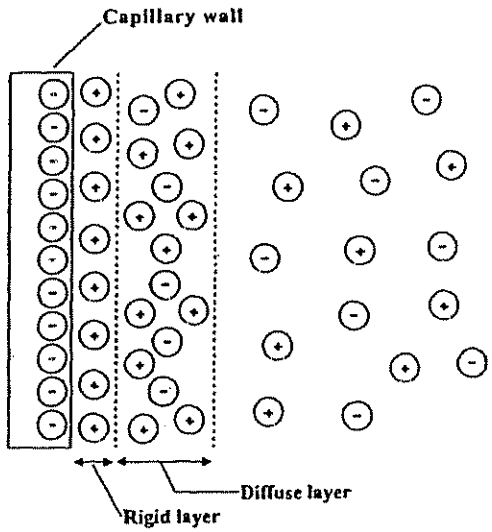


Figure 1.2 *Stern's model of the double-layer charge distribution at a negatively charged capillary wall leading to the generation of a zeta potential and EOF*

solution, creating an electrical double layer and a potential difference (zeta potential) close to the capillary wall, as described according to Stern's model in Figure 1.2. Stern's model for an electrical double layer includes a rigid layer of adsorbed ions and a diffuse layer, in which ion diffusion may occur by thermal motion. The zeta potential is the potential at any given point in the double layer and decreases exponentially with increasing distance from the capillary wall surface.

When a voltage is applied across the capillary, cations in the diffuse layer are free to migrate towards the cathode, carrying the bulk solution with them. The result is a net flow in the direction of the cathode, with a velocity described by

$$v_{EOF} = \left(\frac{\varepsilon_0 \varepsilon \zeta}{4\pi\eta}\right) E \qquad (1.3)$$

where ε_0 is the dielectric constant of a vacuum, ε is the dielectric constant of the buffer, ζ is the zeta potential, η is the viscosity of the buffer and E is the applied electric field. The terms enclosed in brackets equate to the mobility of the EOF (μ_{EOF}).

The relationship between EOF mobility and EOF velocity is analogous to that between electrophoretic mobility and migration velocity. Indeed, the units for EOF mobility are the same as those for electrophoretic mobility.

TAB 4

CAPILLARY ELECTROPHORESIS

THEORY & PRACTICE

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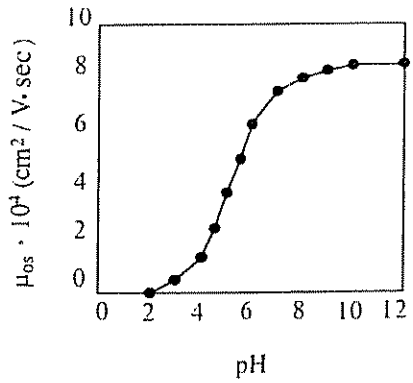

Figure 9 Dependence of electroosmotic mobility, μ_{os}, on buffer pH for the case of a fused-silica capillary that has been prewashed with 1.0 *M* NaOH. Reprinted with permission from Applied Biosystems (1990).

equation (Fahien, 1983),

$$Q = \frac{\pi \Delta P R_t^4}{8 L_{tot} \eta} \tag{50}$$

where ΔP is the pressure drop across the capillary and L_{tot} is its length. Thus, to generate a flow of $3.53 \cdot 10^{-12}$ m^3/sec would require a $\Delta P/L$ of $2.3 \cdot 10^4$ N/m^2/m or 3.4 psi/m. Thus, for this example, with regard to volumetric flow rate, an electrical potential of 30,000 V/m is comparable to a pressure gradient of 3.4 psi/m.

C. Control of Electroosmosis

For many applications it is desirable to be able to manipulate the magnitude of the electroosmotic flow in order to optimize separation performance. Many studies have been conducted describing various methods that can be used to control electroosmotic flow.

In order to control electroosmosis, it is clear from Eqs. (46) or (47) that one must control either the charge density on the capillary wall, the double-layer thickness, or the viscosity of the solution adjacent to the capillary wall. This can be clearly seen if we express Eq. (47) in terms of the double-layer thickness, κ^{-1},

$$\mu_{os} = \frac{\sigma^* \kappa^{-1}(\varepsilon, C_i)}{\eta} \tag{51}$$

where the dependence of κ^{-1} on ε and C_i has been indicated. Therefore, each of the techniques that follow acts by affecting one or the other of these parameters.

Two approaches have been used to control μ_{os} by reducing the double-layer

thickness, κ^{-1}. The first simply uses an increased concentration of electrolytes in the electrophoresis buffer. As can be seen in Eq. (37), this will serve to reduce κ^{-1}. Detailed studies of the effect of varying NaCl concentration on μ_{os} have been reported (Fujiwara and Honda, 1986). A drawback to this approach is that the increased ion concentration will increase the amount of Joule heating within the capillary, thus potentially affecting separation performance. A second approach is to decrease κ^{-1} by decreasing the permittivity of the buffer by the addition of simple organic solvents. Again from Eq. (37), it can be seen that as ε is decreased, κ^{-1} will become smaller. Organic solvents that have been investigated include acetonitrile and methanol (Fujiwara and Honda, 1987). A potential drawback to using organic solvents in the electrophoresis buffer is the large ultraviolet (UV) absorbance background associated with these solvents, which could negatively affect detection sensitivity while using a UV-absorbance detector.

Three main approaches have been examined to control μ_{os} by influencing the surface charge density on the capillary wall, σ^*. The first uses physically adsorbed small cationic molecules to neutralize the charge on the capillary wall. Molecules that have been used for this purpose include cetyltrimethylammonium bromide (Altria and Simpson, 1986), tetradecyltrimethylammonium bromide (Huang *et al.*, 1989), putrescine (Lauer and McManigill, 1986a), and *s*-benzylthiouronium chloride (Altria and Simpson, 1987). Using multivalent ions, one can even reverse the *direction* of the electroosmotic flow using this method (Wiktorowicz and Colburn, 1990). A drawback to this approach is that the cations can potentially bind to the analyte molecule, changing its net charge and thus its electrophoretic mobility. A second approach to influence σ^* is to covalently block the charged silanol groups on the capillary surface. Chemical derivitizing agents that have been used for this purpose include trimethylchlorosilane (Jorgenson and Lukacs, 1983) and (γ-methacryloxypropyl)-trimethoxy silane (Hjertén, 1985). A study of the effect of a number of silanating reagents is given by McCormick (1988). A drawback to this approach is that over time, the covalent bond can hydrolyze, thus allowing the blocking agent to leach off the surface of the capillary, contaminating the buffer and causing μ_{os} to change over time. A third approach used to manipulate σ^* is simply to titrate the charge on the capillary surface. The point of zero charge for fused silica has been found to be approximately at a pH of 2.0 (Churaev *et al.*, 1981). As can be seen from the titration curve in Fig. 9, this does indeed appear to be the case. An obvious drawback of this approach is that if, at the desired pH, the analyte molecule is oppositely charged, a significant amount of interaction between the analyte and the wall can result. However, this method has been effectively exploited for peptide separations at a pH of 2.5 without any apparent wall sticking (Grossman *et al.*, 1989).

The last parameter that can be used to alter μ_{os} according to Eq. (51) is η, the solution viscosity. By adding to the buffer a polymer which adsorbs to the capillary wall, one is able to greatly increase the effective viscosity of the buffer near the capillary–buffer interface. The use of a number of different noncovalently bound polymers for this purpose has been investigated by Herrin *et al.* (1987).

TAB 5

Electrophoresis 2005, *26*, 331–350

Review

End-labeled free-solution electrophoresis of DNA

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DNA is a free-draining polymer. This subtle but "unfortunate" property of highly charged polyelectrolytes makes it impossible to separate nucleic acids by free-flow electrophoresis. This is why one must typically use a sieving matrix, such as a gel or an entangled polymer solution, in order to obtain some electrophoretic size separation. An alternative approach consists of breaking the charge to friction balance of free-draining DNA molecules. This can be achieved by labeling the DNA with a large, uncharged molecule (essentially a hydrodynamic parachute, which we also call a drag-tag) prior to electrophoresis; the resulting methodology is called end-labeled free-solution electrophoresis (ELFSE). In this article, we review the development of ELFSE over the last decade. In particular, we examine the theoretical concepts used to predict the ultimate performance of ELFSE for single-stranded (ssDNA) sequencing, the experimental results showing that ELFSE can indeed overcome the free-draining issue raised above, and the technological advances that are needed to speed the development of competitive ELFSE-based sequencing and separation technologies. Finally, we also review the reverse process, called free-solution conjugate electrophoresis (FSCE), wherein uncharged polymers of different sizes can be analyzed using a short DNA molecule as an electrophoretic engine.

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Abbreviations: ELFSE, end-labeled free-solution electrophoresis; **FSCE**, free-solution conjugate electrophoresis; **CGE**, capillary gel electrophoresis

1 Introduction

The complete sequencing of the Human Genome is certainly one of the greatest achievements of modern science [1, 2]. Capillary gel electrophoresis (CGE) made the completion of this enormous task possible. However, CGE is far from being a perfect analytical tool. For instance, it is well-known that the main physical mechanism responsible for the separation of the long DNA molecules, biased reptation, is limited to providing read-lengths of about 1000 bases because the DNA molecules tend to align with the applied field in the sieving

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matrix during their migration [3]. In addition, the method is rather slow and expensive, and the loading of entangled polymer solutions in small-diameter capillaries is a process that creates many problems. Moving from capillaries to microfluidic devices will not change the latter situation since microchannels are essentially identical to capillaries; in fact, using narrower channels will simply exacerbate the polymer loading issue. Finally, CGE also requires long migration paths (typically on the order of tens of cm) in order for small differential molecular velocities to overcome the band-broadening processes.

A sieving matrix is required for electrophoresis to successfully separate DNA molecules of different sizes because DNA chains act like free-draining polymers during free-solution electrophoresis [3]. Each DNA monomer carries the same electric charge; therefore, the total force F applied to a molecule with M monomers increases linearly with M. If this external force were mechanical (*e.g.*, a sedimentation force), the friction coefficient ζ of the molecule would be that predicted by the Zimm theory of polymer dynamics [4]. Indeed, the different parts of the molecule would interact with each other *via* the fluid (*i.e.*, *via* long-range hydrodynamic interactions), and the random coil molecule would behave like an impermeable sphere of size R_H, the so-called hydrodynamic radius of the random coil. Since $R_H \sim M^{3/5}$ for long, semiflexible polymers [3], this would give $\zeta \sim R_H \sim M^{3/5}$ and a velocity that scales like $v = F/\zeta \sim M^{2/5}$; this hypothetical process would thus lead to a size-dependent velocity and the successful separation of DNA molecules of arbitrary size. Unfortunately, electrical forces generate a different situation, especially when there is salt in the buffer solution, which is always the case in practice. The electric field forces the DNA molecule and its counterions to move in opposite directions. The counterions move through the random coil of the DNA molecule such that the latter is no longer an impermeable sphere; instead, we say that the coil is free-draining. As we will discuss later, this process effectively screens the hydrodynamic interactions between the different parts of the DNA molecule so that the resulting electrophoretic friction coefficient actually scales like $\zeta \sim M$ instead of $\zeta \sim R_H$. This gives rise to a universal electrophoretic velocity $v = F/\zeta \sim M/M \sim M^0$, *i.e.*, the velocity is independent of the size of the DNA molecule and no separation is achieved [3]. Moreover, the local balance between force and friction means that the random coil conformation is not deformed by the migration. This symmetry between force ($F \sim M$) and friction ($\zeta \sim M$) is the unfortunate and unavoidable consequence of the subtle effects taking place during free-flow electrophoresis of large flexible polyelectrolytes in buffer solutions containing salt.

Gel electrophoresis resolves this problem by forcing the DNA molecules to collide with fixed obstacles during their electrophoretic migration [3]. Since longer DNA molecules collide more frequently with the gel fibers, they are slowed down to a larger extent and small molecules elute first, thus providing size-separation. This collision-driven process obviously results in longer elution times, but it also leads to conformational deformation of the DNA since the electrical forces are rather large, and this deformation tends to reduce the size-dependence of the net electrophoretic velocity [3, 5]. In practice, ssDNA molecules longer than about 1000 bases cannot be sequenced because of this molecular deformation effect.

However, gel electrophoresis is not the only way to overcome the free-draining properties of DNA during electrophoresis. In principle, one can also break the balance between force and friction by modifying the DNA at the molecular level. All that one needs to do is to modify the DNA in such a way that its ratio of electric charge to friction coefficient is rendered size-dependent. The only practical way to do this is to increase the friction coefficient of DNA, since it is not possible to substantially modify its average charge density. This idea has probably been around for quite some time; it is clear that many scientists looked at ways to do this back in the 1980s when the Human Genome Project was first conceived. However, it was not possible to test such ideas until the invention of capillary electrophoresis (CE), because free-solution electrophoresis leads to strong heat generation effects when it is not carried out in a narrow-diameter channel. Perhaps the first paper that specifically mentions this idea is that of Noolandi in 1992 [6]; however, the paper is purely speculative and does not provide experimental data or theoretical predictions. Moreover, this paper suggests attaching an object with a negative charge to ssDNA molecules prior to free-solution electrophoresis; most likely, this would be an inefficient way to restore a size-dependent DNA velocity, since the object would increase both the net force and the net friction on the ssDNA molecules.

The first paper to examine this concept quantitatively was published in 1994 by Mayer, Slater, and Drouin [7]. The theory developed by these authors made remarkable predictions and the name end-labeled free-solution electrophoresis (ELFSE) was coined for the process wherein an uncharged label is attached to the end of the DNA molecule in order to increase its friction coefficient without affecting its total charge. The paper mentions the possibility of sequencing more than 1000 or even 2000 bases in less than 1 h without the need for a sieving matrix. As we shall see later, the theory used by these authors needs major revisions, but their general conclusions are still valid.

TAB 6

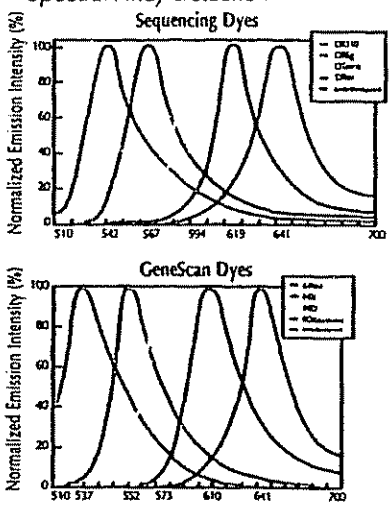
ABI PRISM® 3700 DNA Analyzer

System Profile

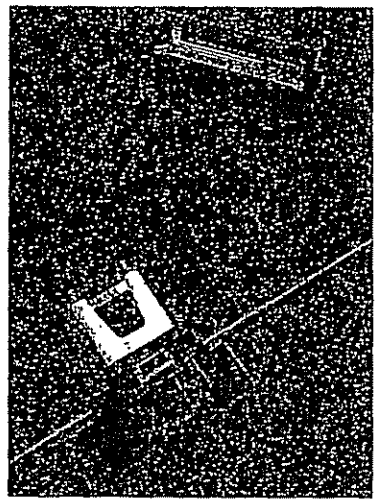
September 1998

PE Applied Biosystems

• *Spectral Array Detection*



Emission Spectra of dyes used with 3700 analyzer.



User installed 3700 capillary array in load bar and sheath flow cuvette (instrument fixtures). Laser beam simulated for illustration.

Once the emitted fluorescent light is collected and dispersed across the CCD, this data is transferred to the instrument computer where chemometric algorithmic processing transforms it into 4-dye or 5-dye electropherograms. This method of collecting and imaging light has several advantages. It greatly reduces the experimental noise in the dye electropherogram due to the spectral oversampling, and it avoids time interpolation problem by simultaneously collecting all the colors. Finally, this method provides versatility to use new chemistries and dye sets as they become available without requiring changes in the optical hardware, as is the case with systems using fixed optical filters.

High Performance Capillaries with Dynamically Coating Polymer

In capillary electrophoresis, the ionic double-layer at the capillary wall surface must be neutralized to suppress electro-osmotic flow. Suppression of electro-osmosis enables the DNA to migrate from the cathode toward the anode without the influence of bulk fluid flow.

Neutralizing the surface of the capillary wall can be done by a variety of methods. Most methods involve applying a bonded phase during the manufacturing process of the capillary bundles. These permanent coatings are problematic because they degrade over time and become fouled by sample contaminants. On systems using capillaries with a bonded phase, the capillaries must be discarded once the fouling inhibits their performance, resulting in capillaries that last fewer than 100 runs.

At PE Applied Biosystems, the ion boundary at the surface of the capillaries is masked by chemiabsorption of a specially formulated polymer. This polymer, 3700 POP-6™, which is also used as the separation matrix, is a low viscosity, flowable polymer, that enables the system to pump polymer in and out of capillaries rapidly and automatically. Because the polymer is absorbed to the capillary walls, the 3700 system can use bare silica capillaries. The benefit of using this approach is that the capillary surface can be returned to bare silica using an acid wash, thus removing any fouling contaminants which were not pumped out with the polymer. This acid wash is a procedure that would destroy bonded phase capillaries. An acid wash protocol can be run automatically on the 3700 analyzer as scheduled by the user. Bare silica capillaries and 3700 POP-6 polymer eliminate the problems associated with bonded phase, extending capillary lifetime. Studies performed at PE Applied Biosystems, as well as independent testimonials from 310 analyzer users, indicate that the 3700 system capillary arrays will last for greater than 300 runs with periodic, automated washes.

The 3700 system capillary array is a user-replaceable array of 96 primary and 8 reserve 50 µ id, long-lifetime capillaries. For protection of the capillary array investment, the 3700 system can be programmed to bypass an unusable capillary and use a reserve in its place. Arrays are available in either 32 cm or 50 cm lengths. The different lengths allow flexibility in managing run duration.